1α,25-Dihydroxycholecalciferol and Cyclosporine Suppress Induction and Promote Resolution of Psoriasis in Human Skin Grafts Transplanted on to SCID Mice

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Accumulating evidence has emphasized the importance of immunocompetent cells in determining the psoriatic phenotype. We have investigated the effect of 1α,25-dihydroxycholecalciferol, the naturally occurring active form of vitamin D_3, cyclosporine A, and interleukin-10 on the phenotype of human psoriatic skin xenotransplants. First, psoriatic skin transplants were injected with either 1α,25-dihydroxycholecalciferol, cyclosporine A, or interleukin-10. Second, we determined the ability of autologous lymphocytes, activated *in vitro* using staphylococcal enterotoxin B and interleukin-2 and then exposed to either 1α,25-dihydroxycholecalciferol or cyclosporine A, to induce psoriatic lesions if they were injected into the dermis of uninvolved skin grafts. We found that injections into transplanted psoriatic plaques of either 1α,25-dihydroxycholecalciferol or cyclosporine A, but not interleukin-10, resulted in a consistent reduction in the clinical and histologic score of psoriasis with remission towards uninvolved psoriatic skin. Injection of activated immunocytes into symptomless psoriatic skin grafts, changed the grafts towards plaque-type psoriasis with silvery scale, parakeratosis, elongated rete pegs, acanthosis, and dermal angiogenic reaction. In contrast, if activated immunocytes were exposed to 1α,25-dihydroxycholecalciferol or cyclosporine A prior to injection, only minimal changes occurred. It was determined that neither staphylococcal enterotoxin B and interleukin-2 activation by itself, nor the drugs investigated, changed the CD4/CD8 ratio of activated ($CD25^+$) cells. Our results are consistent with the hypothesis that psoriasis may be induced by activated T lymphocytes, and indicate that novel immunomodulatory drugs can serve to inhibit the pathogenetic ability of immunocytes in psoriasis.

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Immune activation is thought to play a crucial part in the pathogenesis of psoriasis (Bos *et al*, 1983; Baker *et al*, 1984), and evidence that the psoriatic phenotype can be induced and maintained by activated T cells is growing (Nickoloff and Wrone-Smith, 1998). Furthermore, it has been demonstrated that the cytokine release in lesional and nonlesional psoriatic skin is of T helper (Th) 1 type (Nickoloff, 1991; Uyemura *et al*, 1993; Schlaak *et al*, 1994). Th1 clones are characterized by strong cellular immune responses and elevated interleukin (IL) –2, interferon-γ and tumor necrosis factor-α cytokines. Human T cell clones tend to maintain their Th1 or Th2 phenotype, but IL-2, IL-4, interferon-γ, IL-10, and IL-12 can variably modulate the proliferation rate and the amount of cytokines produced by these clones (Del Prete *et al*, 1994).

The pathophysiologic mechanism of psoriasis has not yet been established, but several reports suggest that streptococcal or staphylococcal-derived superantigen may play a part by binding to major histocompatibility complex class II bearing antigen-presenting cells (Langerhans cells, dermal dendritic cells, and locally infiltrating macrophages) and, if the appropriate Vβ-bearing T lymphocytes were present, result in memory T cell activation and proliferation (Leung *et al*, 1995; Valdimarsson *et al*, 1995, 1997). Recently it has been hypothesized that these superantigen activated $CD4^+$ T cells can re-enter the circulation and home for the skin where a subset eventually recognizes an autoantigen, resulting in proliferation and lymphokine production, leading to epidermal stem cell growth and characteristic features of a psoriatic plaque (Sigmundsdóttir *et al*, 1997).

Recognition that in psoriasis there is a Th1 response has encouraged us to study the effect of immunomodulating drugs. Research into new strategies for immunotherapeutic intervention in psoriasis has, however, been hampered by the lack of an appropriate animal model. Severe combined immunodeficient (SCID) mice transplanted with psoriatic human skin have previously been shown to be a useful experimental model of psoriasis (Nickoloff *et al*, 1995; Wrone-Smith and Nickoloff, 1996; Boehncke *et al*, 1997); furthermore, this model offers possibilities of studying therapies targeted directly to the skin immune system.

Clinical studies have shown that oral or topical administration of calcitriol (1α,25-dihydroxycholecalciferol), the 1α-hydroxylated and biologically active metabolite of vitamin D_3, is effective in the treatment of psoriasis (Morimoto and Kumahara, 1985; Morimoto *et*

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Abbreviations: $1,25(OH)_2D_3$, 1α,25-dihydroxycholecalciferol; PN, symptomless psoriatic skin; PP, psoriatic plaque; SCID, severe combined immunodeficiency; SEB, staphylococcal enterotoxin B.

al, 1986). The more rapidly metabolized synthetic analogs (calcipotriol, KH1060 and tacalcitol), with less calcemic effects *in vivo*, are effective and safe therapies for psoriasis (Morimoto and Kumahara, 1985; Morimoto *et al*, 1986; Kragballe *et al*, 1988, 1991, 1994). Both 1α,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and the synthetic analogs have potent immunoinhibitory properties on activated $CD45R0^+$ T cells *in vitro* (Müller and Bendtzen, 1992). Although $1,25(OH)_2D_3$ and the synthetic analogs may act by inhibiting keratinocyte proliferation, their immunoregulatory properties in the skin may also be important (de Jong and van de Kerkhof, 1991; Mozzanica *et al*, 1994; Lu *et al*, 1996) and, in many respects, appear to be similar to cyclosporine A (Meehan *et al*, 1992; Mozzanica *et al*, 1993; Kurimoto *et al*, 1994). Both compounds act on T lymphocytes during initial activation by antigen-presenting cells and inhibits accumulation of mRNA for IL-2, interferon-γ, granulocyte-macrophage colony-stimulating factor, and IL-12 (Lemire *et al*, 1985; Rigby *et al*, 1990a; Lemire, 1995). They both appear to select the T helper cell by inhibiting lymphokine production at a genomic level and inhibit the generation of cytotoxic and natural killer cells. The vitamin D receptor has been identified in almost all cell types studied (Stumpf *et al*, 1979; Berger *et al*, 1988), including human epidermal Langerhans cells (Dam *et al*, 1996).

In this study (PP model), the effectiveness of two standard therapies ($1,25(OH)_2D_3$ and cyclosporine) were compared against IL-10. The rationale for selecting IL-10 derives from results in mice showing that IL-10 can selectively inhibit Th1 cytokine production. IL-10 is produced by keratinocytes, monocytes/macrophages, and Th2 lymphocytes and is conspicuous by its absence in psoriasis (Nickoloff *et al*, 1994).

MATERIALS AND METHODS

Human skin/SCID mouse chimera and tissue processing Keratome biopsies ($7 \times 2 \times 0.05$ cm keratome containing both dermis and epidermis) were obtained from clinically symptomless skin (PN; n = 5) on the lower back or buttocks, or from psoriatic plaques (PP; n = 6). Prior to the procedure, the skin area was defined and infiltrated with 1% lidocaine with epinephrine 1 : 200. The psoriatic patients included six males and five females aged 34–66. This study was approved by the University of Michigan Human Subjects Committee (Ann Arbor, MI), and informed consent was obtained from each patient before the procedure. The keratomes were transferred to Earle's Balanced Salt Solution (GIBCO, Grand Island, NY) supplemented with 400 U penicillin per ml, 400 μg streptomycin per ml, and 4 mg gentamicin per ml (GIBCO) and immediately transferred to the cold room at 4°C until transplanted (≤ 24 h). Immediately before orthotopic transplantation on to the flank area of 6–8 wk old anesthetized (intraperitoneal injection of 1.56 mg phenobarbital) C.B-17 SCID mice (Taconic Farms, Germantown, NY), human skin xenografts ($1.7 \times 2.2 \times 0.05$ cm) were cut from the keratomes, then the grafts were sutured to each SCID mouse with absorbable 6-0 Vicryl Rapide suture (Ethicon, Somerville, NJ) and covered with Xeroform dressings (Kendall, Mansfield, MA) for 1 wk. All animal-related procedures were performed at the Unit for Laboratory Animal Medicine (ULAM), and the mice were kept under pathogen-free conditions throughout the study. Animals transplanted with psoriatic plaque (PP model, **Fig 1**) were then randomized to the following treatments: intradermal administration of either 63 ng $1,25(OH)_2D_3$ (Leo Pharmaceutical Products, Ballerup, Denmark), 0.15 mg cyclosporine A (Sandoz, Basel, Switzerland), or 990 U IL-10 (R&D Systems, Minneapolis, MN) diluted in 250 μl sterile phosphate-buffered saline (PBS) were injected intradermally into the xenografts using a 30½ G needle (Becton Dickinson, Franklin Lakes, NJ). Within 3–4 wk the mice were killed by CO_2 asphyxiation, and 4 mm punch biopsies (Acu Punch; Acuderm, Ft Lauderdale, FL) were obtained from each xenograft. Biopsies were fixed in 10% neutral-buffered formalin for paraffin embedding, and/or transferred to cryotubes and snap frozen in liquid nitrogen, and stored at –80°C.

Activation of autologous immunocytes Peripheral blood mononuclear cells (PBMC) from psoriatic patients (and two normal controls included for flow cytometry study) were isolated by density centrifugation from heparinized blood diluted 1:1 with Hank's (GIBCO) overlayered on Histopaque 1.077 mg per ml (Sigma, St Louis, MO) and washed twice in RPMI-1640 (GIBCO). Immunocytes ($1–2 \times 10^6$ cells per ml) were then transferred to serum-RPMI (15% heat-inactivated autologous serum in RPMI-1640, 2 mM L-glutamine, 100 U penicillin per ml, 100 μg streptomycin per ml, and 1 mg gentamicin per liter (GIBCO) containing 1 μg Staphylococcal enterotoxin B (SEB) per ml (Toxin Technologies, Sarasota, FL) and 20 U per liter human IL-2 (Boehringer Mannheim Biochemicals, Indianapolis, IN) and cultured (37°C, 5% CO_2) in six well tissue culture clusters (Costar, Cambridge, MA) for 48 h. The cells were pipetted off the plates, gently washed in cold medium to release adherent cells, washed twice in RPMI-1640 and reincubated for 48 h ($1–2 \times 10^6$ cells per ml) in serum-RPMI with either: $1,25(OH)_2D_3$ (10^{-7} M), cyclosporine A (1 μg per ml) or diluent control (PBS). In pilot experiments, several different concentrations for each drug (including IL-10 200 U per ml) were explored to arrive at the final concentration for this study. Finally the cells were removed, washed twice in RPMI-1640, resuspended ($10–15 \times 10^6$ cells per ml) in PBS (GIBCO), and transferred to 1 ml syringes (Becton Dickinson) for injection into the PN grafts (**Fig 2**).

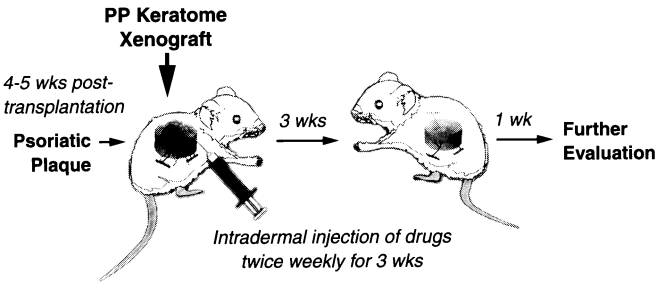

Figure 1. Pharmacologic treatment of psoriatic plaques transplanted on to SCID mice. Schematic overview of the protocol used to determine the effectiveness of various treatments in promoting resolution of psoriatic plaques (PP model). Keratome biopsies (including epidermis and dermis) of psoriatic plaques were transplanted on to SCID mice and allowed to engraft for 4–5 wk. Then, the drugs under investigation were injected twice weekly for 3 wk. One week following the last injection, the mice were killed and biopsies were obtained for further evaluation.

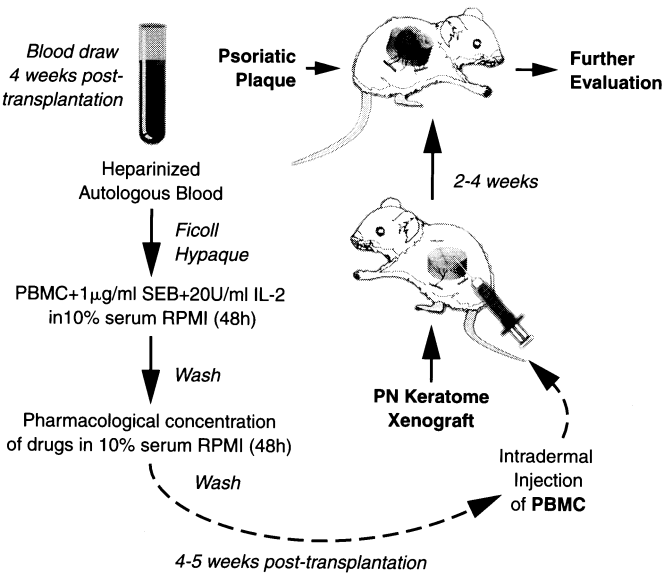

Figure 2. Phenotypic conversion of uninvolved psoriatic skin into a psoriatic plaque (PN model). Schematic overview of protocol used to induce/suppress induction of psoriatic plaques in uninvolved (PN) keratomes from psoriatic patients, transplanted on to SCID mice. Four to 5 wk after xenotransplantation, PBMC were isolated from autologous blood, activated using SEB and IL-2 for 48 h, re-incubated in medium containing the drugs investigated, then injected intradermally in the PN grafts. After 3–4 wk, the phenotype of the grafts injected with activated cells re-incubated in control medium changed towards characteristic plaque-type psoriasis; the mice were then killed and biopsies obtained for further evaluation.

Flow cytometry of immunocytes Freshly isolated PBMC from two psoriatic patients and two normal controls were analyzed. PBMC were activated as previously described; and samples of freshly isolated PBMC, immunocytes activated using SEB + IL-2, and pharmacologically modified activated cells were stained for triple-color flow cytometry. Cells were washed in PBS + 0.2% bovine serum albumin (BSA), and stained at 4°C for 20 min with a mixture of anti-CD25-FITC (DAKO A/S, Glostrup, Denmark, 10 μg per ml), anti-CD4-RPE (DAKO, 5 μg per ml) and, anti-CD8-PE-Cy5 (Immunotech, 10 μg per ml) or relevant isotype matched controls [IgG1 – Cy5 (DAKO) and IgG1 – RPE + IgG1 – FITC (Coulter)]. The cells were then washed in PBS + 0.1% BSA, fixed in 0.1% paraformaldehyde and analyzed (Coulter, Elite).

Clinical assessment and light microscopic evaluation During the injection phase and follow-up phase of the study the severity of the psoriatic lesions were assessed twice weekly by two independent investigators for each of the following clinical signs: scaliness (S), induration (I) and, erythema (E). Each parameter was scored using the three-point scale: 0 = complete lack of cutaneous involvement; 1 = slight involvement; 2 = moderate involvement; 3 = severe involvement. The following formula was used to calculate a semiquantitative score: 1/3 (S + I + E). On this scale ranging from 0 to 3, a maximal score of three represents severe scale, induration, and erythema of the psoriatic xenografts. At the end of the study, when the grafts were removed, another semiquantitative scale (0–3) was used to indicate the extent of new vessel formation (angiogenesis) that could be seen between the xenograft and the underlying fascia muscularis (**Fig 3**). A calibrated microscope eyepiece (Nikon microscope) was used for estimating the epidermal thickness in the vertical cryosections (average of five random measurements from stratum corneum to the deepest part of the rete-pegs).

Statistical analysis Skin from each individual has been transplanted to at least 12 mice, depending on the size of the skin grafts obtained. The mice were randomized among the treatments. Average for the repeats was calculated, then the data from n patients were summarized as a mean ± SEM. Statistical differences were calculated using Turky's studentized range test for multiple comparisons and considered significant at the 0.05 level.

RESULTS

Injection into psoriatic xenografts of cyclosporine A or $1,25(OH)_2D_3$, but not IL-10, promote resolution of psoriasis (PP model) In this study, psoriatic plaques were transplanted to SCID mice (**Fig 1**). Previously, this model has been validated, and it has been determined that human psoriatic grafts can be successfully transplanted to SCID mice and retain clinical, light microscopic, and immunohistochemical characteristics when compared with pretransplanted skin (Nickoloff *et al*, 1995). Injections of cyclosporine A 0.15 mg or $1,25(OH)_2D_3$ 63 ng twice weekly for 3 wk into psoriatic xenotransplants changed the severity of scaliness, induration, and erythema as indicated in a semiquantitative score of psoriasis (**Fig 4*b***). As early as day 18, injections of cyclosporine A 0.15 mg or $1,25(OH)_2D_3$ 63 ng are more effective than injections with vehicle control (*PBS*) or IL-10 990 U in promoting resolution (**Fig 4*a***). At the end of this study the mice were killed and biopsies were obtained (**Fig 1**). Measurement of epidermal thickness confirmed the clinical observations that cyclosporine A 0.15 mg injections are significantly more effective than vehicle control injections (**Fig 4*c***). Furthermore, both $1,25(OH)_2D_3$ 63 ng and cyclosporine A 0.15 mg was able significantly to inhibit the angiogenic tissue response seen in grafts injected with vehicle control or untreated grafts (**Fig 4*d***).

Administration of $1,25(OH)_2D_3$ 63 ng is almost as effective as cyclosporine A 0.15 mg in promoting resolution of psoriasis. In contrast IL-10 990 U injections are not causing any detectable decrease in epidermal thickness, but rather produced a slight increase in semiquantitative scores of psoriasis and angiogenesis (**Fig 4*b*, *d***).

Light microscopic characteristics of biopsies obtained from vehicle control (PBS) injected skin were pathognomonic of psoriasis: hyperkeratosis, parakeratosis, acanthosis with elongated rete pegs, and a conspicuous mononuclear cell infiltrate (**Fig 5*A***). The histologic evaluation indicates near-complete resolution of

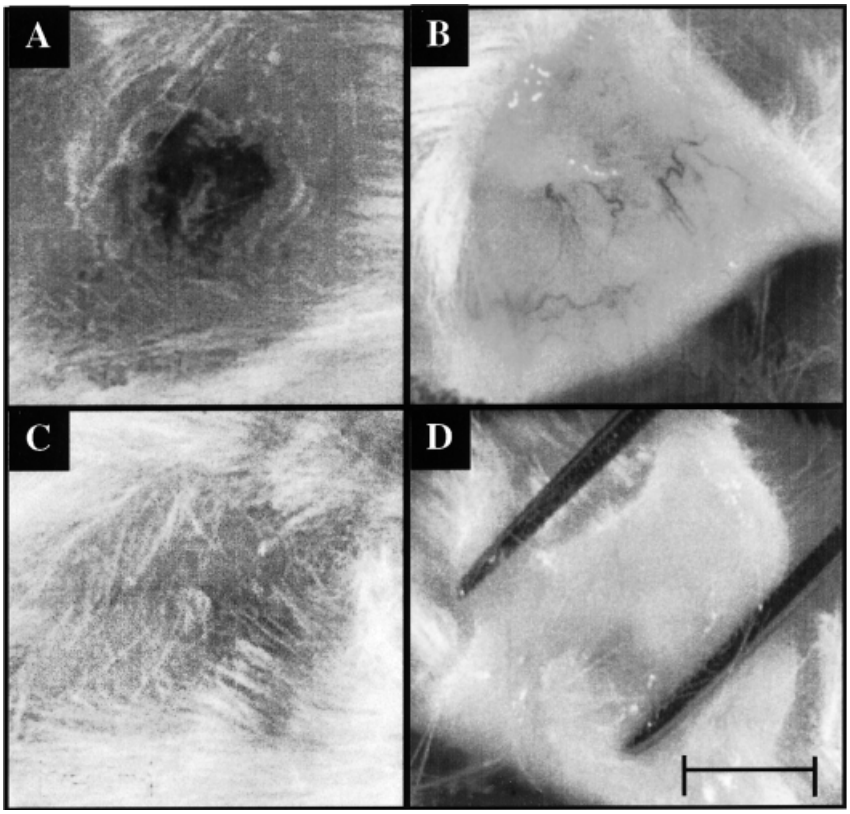

Figure 3. Clinical appearance and blood vessel formation at the end of the study distinguish grafts injected with immune deviated cells from controls (PN model). Graft injected with activated (*PBS* control) cells (*A*, *B*), and graft injected with 48 h cyclosporine A (*CsA*) pulsed activated cells (*C*, *D*). *Scale bar*: 1cm.

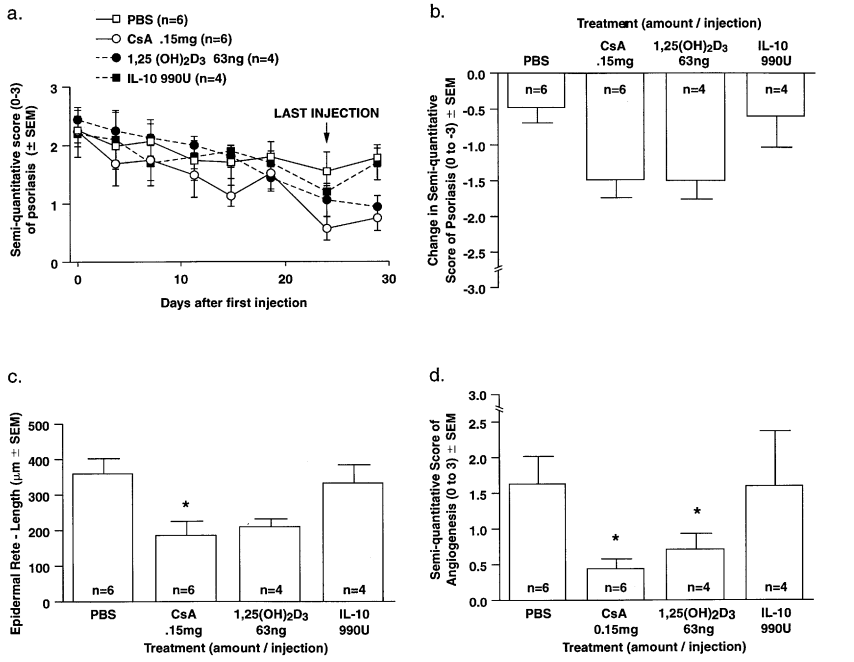

Figure 4. Local injections twice weekly for 3 wk of cyclosporine A 0.15 mg or $1,25(OH)_2D_3$ 63 ng, but not IL-10 990 U, into psoriatic xenografts promotes resolution of psoriasis (PP model). (*a*) Clinical assessments during the injection phase and follow-up phase of the study were indicated on a semiquantitative scale of psoriasis ranging from 0 to 3, a maximal score of three represents severe scale, induration, and erythema of the psoriatic xenografts (for details see *Materials and Methods* and **Figs 1** and **3**). (*b*) At the end of the study, the overall changes from baseline in clinical score of psoriasis were calculated (0 to −3). Further evaluation was made after the mice had been killed at the end of the study. Blood vessel formation (**Fig 3**) was indicated on a semiquantitative score of angiogenesis ranging from 0 to 3 (*d*). Furthermore, hematoxylin and eosin stained sections (**Fig 5**) from formalin fixed and paraffin embedded biopsies were used to determine the length of the epidermal rete pegs (five random selected measurements on each microscopy slide) (*c*). *Error bars*, mean ± SEM (*$p < 0.05$ *versus* control).

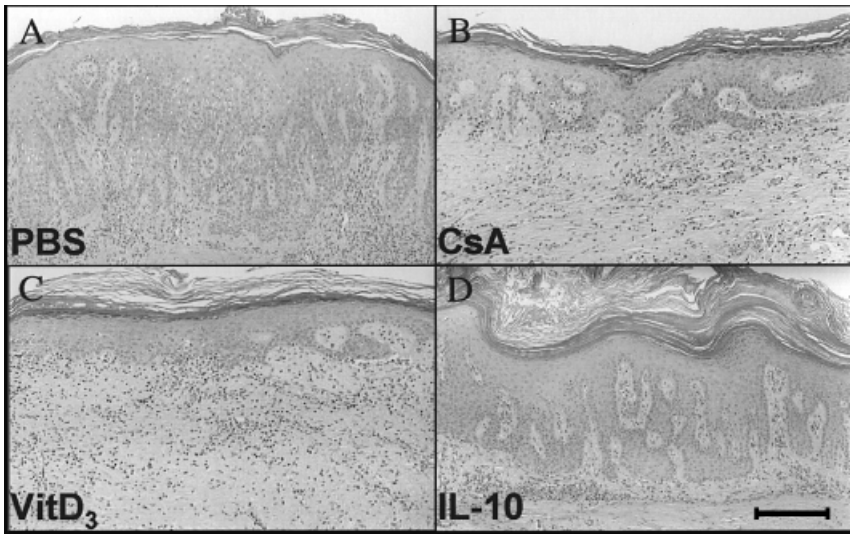

Figure 5. Histologic evaluation indicates resolution of psoriasis after injections of cyclosporine A 0.15 mg or $1,25(OH)_2D_3$ 63 ng but not IL-10 990 U into psoriatic xenografts (PP model). For details see *Materials and Methods* and **Fig 2.** Hematoxylin and eosin staining of biopsies from psoriatic xenografts injected with PBS control (*A*), cyclosporine. A (*B*), $1,25(OH)_2D_3$ (*C*) and, IL-10 (*D*). *Scale bar*: 100 μm.

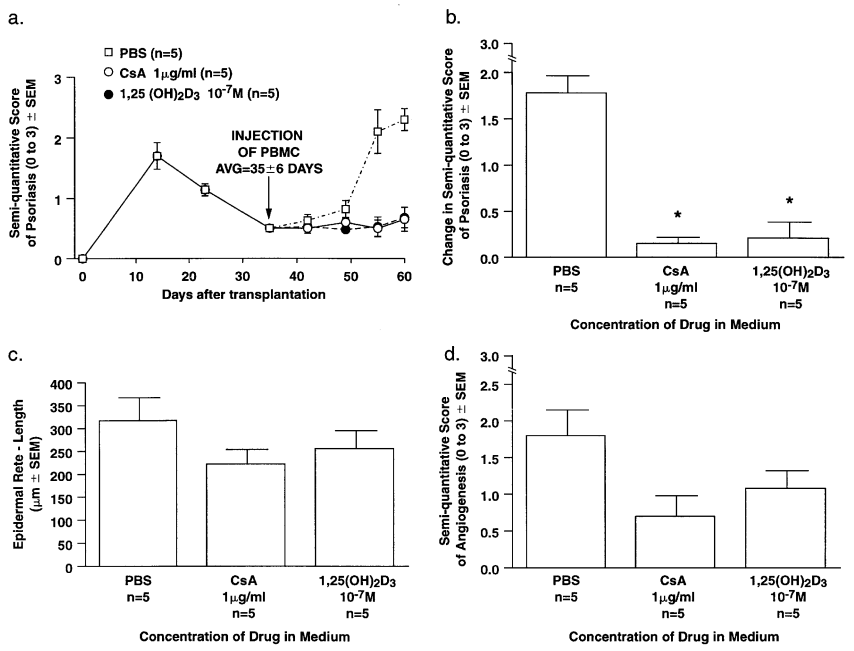

Figure 6. Pharmacologic modulation of activated PBMC with $1,25(OH)_2D_3$ or cyclosporine A prior to injection in uninvolved psoriatic xenografts suppress the induction of psoriasis (PN model). (*a*) Clinical assessments during the engraftment and induction phases of the study are indicated on a semiquantitative scale of psoriasis (0–3) where a maximal score of three represents severe scale, induration, and erythema of the psoriatic xenografts (see *Materials and Methods* and **Figs 2** and **3** for details). (*b*) At the end of the study, the overall changes from baseline in clinical score of psoriasis were calculated (0–3). Further evaluation was made after the mice had been killed at the end of the study. Blood vessel formation (**Fig 3**) was indicated on a semiquantitative score of angiogenesis ranging from 0 to 3 (*d*). Furthermore, hematoxylin and eosin stained sections (**Fig 7**) from formalin-fixed and paraffin-embedded biopsies were used to determine the length of the epidermal rete pegs (five random selected measurements on each microscopy slide) (*c*). *Error bars*, mean ± SEM (*$p < 0.05$ *versus* control). Note: data points for $1,25(OH)_2D_3$ and cyclosporine A are almost congruent after 50 d (*a*).

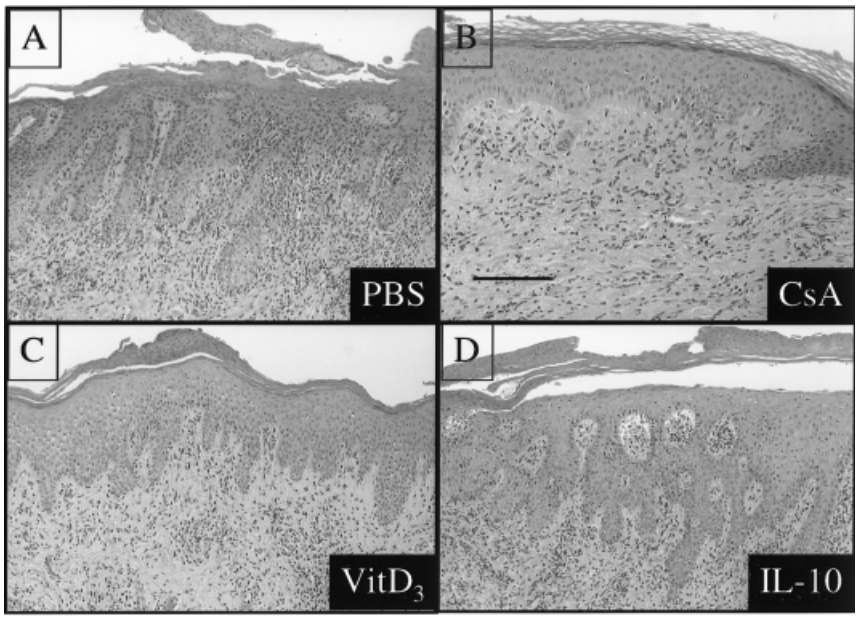

Figure 7. Histologic evaluation confirmed that 48 h pulse of immunocytes with $1,25(OH)_2D_3$ or cyclosporine A prior to injection in the grafts could suppress the induction of psoriasis (PN model). For details see *Materials and Methods* and **Fig 2.** Hematoxylin and eosin staining of biopsies from psoriatic xenografts injected with activated immunocytes pulsed for 48 h prior to injection. Pretreatments: (*A*) PBS control; (*B*) cyclosporine A; (*C*) $1,25(OH)_2D_3$; and (*D*) IL-10 (200 U per ml). *Scale bar*: 100 μm.

psoriasis and reformed granular layer after injections of cyclosporine A 0.15 mg or $1,25(OH)_2D_3$ 63 ng. In contrast, injections of IL-10 990 U inclined acanthosis and hyperkeratosis (**Fig 5*B–D***).

Pharmacologic modulation of activated PBMC with $1,25(OH)_2D_3$ or cyclosporine A prior to injection in uninvolved psoriatic xenografts suppress induction of psoriasis (PN model) In this study, uninvolved psoriatic skin (PN) was transplanted to SCID mice, then activated autologous immunocytes were pharmacologically modified before they were reintroduced in the xenotransplants to induce/suppress induction of the psoriatic phenotype (**Fig 2**). The human skin transplants contracted by 30–40%, and became hyperpigmented with scale production. Moreover, the epidermis thickened after transplantation (data not shown). Approximately 12 d after the transplantation, the scale production had reached a maximum; however, 35 ± 6 d after the transplantation the clinical appearance of the grafts was almost indistinguishable from that of the transplanted skin (**Fig 6*a***).

Intradermal injection of activated immunocytes resulted in a dramatic increase in the semiquantitative score of psoriasis (severity of scaliness, erythema, and infiltration) that morphologically resembled a full-fledged psoriatic lesion. In contrast, if activated autologous immunocytes were exposed to 1 μg cyclosporine per ml or $1,25(OH)_2D_3$ 10^{-7} M prior to intradermal injection, the clinical score of psoriasis remained unchanged throughout the remaining study period (**Fig 6*a***). At the end of the study, the mice were killed and biopsies were obtained. Overall changes in semiquantitative scores of psoriasis were significantly lower if the immunocytes had been exposed to either 1 μg cyclosporine per ml or $1,25(OH)_2D_3$ 10^{-7} M prior to injection (**Fig 6*b***). Exposure of immunocytes to $1,25(OH)_2D_3$ 10^{-7} M is almost as effective as 1 μg cyclosporine A per ml in suppressing the induction of psoriasis. Semi-quantitative scores of angiogenic tissue response and measurements of the epidermal thickness correlated with the clinical data but were statistically insignificant (**Figs 6*c*, *d*** and **Fig 3**).

Light microscopic characteristics of biopsies obtained from xenografts injected with unmodified activated immunocytes revealed pathognomonic changes of psoriasis: hyperkeratosis, parakeratosis, acanthosis with elongated rete pegs, and a mononuclear cell infiltrate (**Fig 7*a***). In contrast, if the grafts had been injected with immunocytes exposed to either 1 μg cyclosporine per ml or $1,25(OH)_2D_3$ 10^{-7} M before injection, hyperkeratosis, acanthosis, and parakeratosis were less pronounced. In all the biopsies there were lymphocytes around the superficial vascular plexus, indicating that the injected immunocytes were present in the graft throughout this study (**Fig 7*A–D***).

Flow cytometry analysis of IL-2 receptor (CD25) expression on $CD4^+$ and $CD8^+$ cells from psoriatic patients and normal controls indicated that both T cell subtypes were activated by SEB + IL-2, and the CD4/CD8 ratio remained unchanged after pharmacologic modulation (PN model) We have addressed the question whether exposure of SEB + IL-2-activated-immunocytes to either cyclosporine or $1,25(OH)_2D_3$ (see *Materials and Methods* for details) would skew the activation process, by changing the CD4/CD8 ratio of activated ($CD25^+$) cells. $CD4^+$ and $CD8^+$ cells from psoriatic patients and normal controls all increased their CD25 expression after activation with SEB + IL-2. Furthermore, in both groups, neither the CD4/CD8 ratio nor the number of activated $CD4^+$ or $CD8^+$ cells was changed by the drugs investigated (**Fig 8**).

DISCUSSION

Over the past several years a number of investigators have utilized different animal models of psoriasis, contributing to our understanding of the role of immunocompetent cells (Nickoloff *et al*, 1995; Gilhar *et al*, 1997; Nickoloff and Wrone-Smith, 1997; Schon *et al*, 1997; Nickoloff, 1999) and growth factors in psoriasis. Whereas transgenic mice have not been proved to have a psoriatic phenotype (Cook *et al*, 1997), engraftment of psoriatic skin on to SCID mice provides an opportunity to study psoriasis (Wrone-Smith and Nickoloff, 1996; Boehncke *et al*, 1997). As psoriasis is solely a disease of the human skin, it appears that models based on xenotransplantation of skin from psoriatic patients may be advantageous because they involve the study of human tissue. The main objective of this project was to extend our earlier observations in which psoriasis could be created with a high degree of fidelity using this SCID mouse model, to validate this model from a pharmaceutical perspective. Using two known anti-psoriatic treatments (i.e., cyclosporine A and $1,25(OH)_2$ D_3), and a third agent of unknown clinical efficacy (i.e., IL-10), the reliability of the SCID mouse xenograft system was explored in both the PP as well as the PN models. In this study, we have demonstrated that the psoriatic skin SCID mouse model (PP model) can efficiently be used to study the efficacy of various therapeutic agents. Initial assignment of the therapies to individual mice were blinded for the investigator; hence it was necessary to standardize the administration of the therapeutic agents. Intradermal injection was chosen to deliver the drugs directly into the grafts. Although the drugs studied

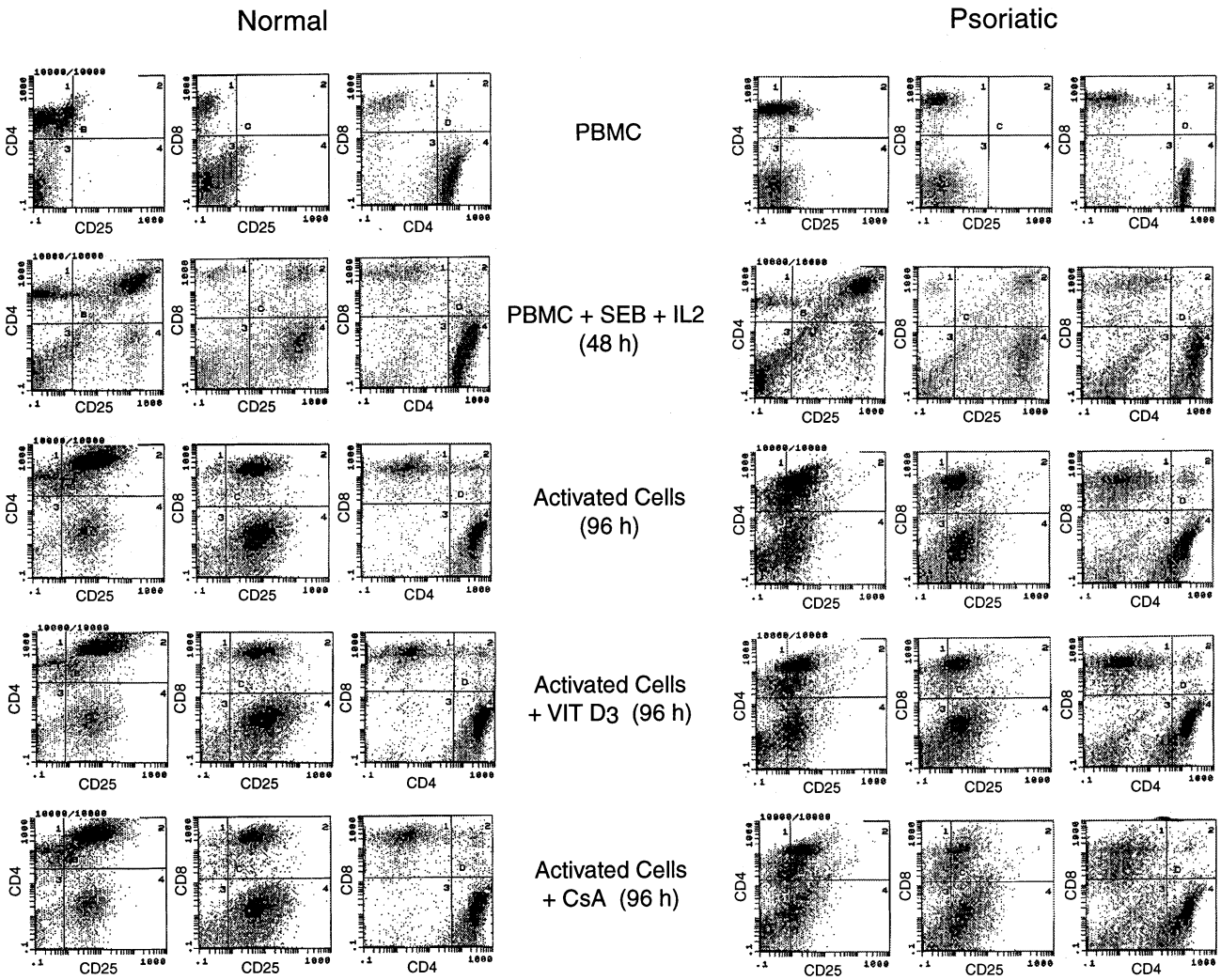

Figure 8. Flow cytometry analysis of IL-2 receptor (CD25) expression on $CD4^+$ and $CD8^+$ cells from psoriatic patients and normal controls indicated that both subtypes were activated by SEB + IL-2, and that the CD4/CD8 ratio remained unchanged after pharmacologic modulation. Cytofluorometric analysis of immunocytes from normal controls (*left panel*) and psoriatic patients (*right panel*). Cells were analyzed at different stages during activation and preincubation before injection into human skin xenografts (**Fig 2**): freshly isolated peripheral blood mononuclear cells, PBMC; PBMC activated with staphylococcal enterotoxin B (SEB) and IL-2, PBMC + SEB + IL-2 (48 h); activated untreated cells, Activated Cells (96 h); activated cells pulsed with $1,25(OH)_2D_3$, Activated Cells + VIT D_3 (96 h) and activated cells pulsed with cyclosporine A, Activated Cells + cyclosporine A (96 h). For details see *Materials and Methods*.

all have different pharmacokinetic data it was decided to give the injections twice weekly. Assuming a distribution volume equal to the total body volume, the dosage effective for alteration of immune parameters for each drug was determined from previously published *in vitro* and *in vivo* data indicating the optimal concentrations to inhibit the cellular immune response or cytokine production (Rigby *et al*, 1990b; Cooper *et al*, 1992). The injected dose of 0.15 mg per injection (5.0 mg per kg) twice weekly was selected because the average dose per week would then be 1.43 mg per kg per d, or in the same range as the clinically used doses for psoriasis. The skin levels of cyclosporine A after oral administration has been studied by Ellis *et al* (1991), and it has been determined that oral dosing levels of cyclosporine A from 3 to 14 mg per kg per d resulted in tissue levels in psoriatic skin ranging from 1.0 to 2.9 μg per ml per wet weight of tissue. Based on these and other results it has been estimated that the clinical use of cyclosporine A for psoriasis results in skin concentrations in the range 1–2 μg per ml per wet weight of tissue (almost the same concentration as in the *in vitro* PN model). The effects of $1,25(OH)_2D_3$ on immune functions and maturation of monocytes have been observed at concentrations that are 10–100 times higher than the physiologic plasma levels (40–160 pg per ml) (Müller *et al*, 1988). It is, however, likely that the concentration in the microenvironment of the activated and $1,25(OH)_2D_3$-producing monocytes may be much higher than those in the circulation. *In vitro* cultures of monocytes may contain concentrations of $1,25(OH)_2D_3$ up to 70 times higher than normal plasma levels, and this has led to speculation that $1,25(OH)_2D_3$ has a role as an autocrine and paracrine modulator of immune functions. In this study we have used $1,25(OH)_2D_3$ at a supraphysiologic but non toxic concentration known to be effective for immune modulation.

Separate experiments, in which the drugs were administered as intraperitoneal injections, were performed to test the hypothesis that the drugs were redistributed after intralesional injection. Although the number of observations were not large enough to be significant, the intraperitoneal results were congruent with the results seen after intradermal injections. Therefore, it was assumed that the drugs were redistributed after injection, and then eliminated according to their specific pharmacokinetics. Only limited data are available regarding the toxicology of cyclosporine A, $1,25(OH)_2D_3$ (Mortensen *et al*, 1993), and IL-10 in studies of rodents. In initial studies we have weighed each mouse at baseline

and at the end of the study. We did not find any statistically significant difference in weight between the groups, thus arguing against the theory that the therapies caused metabolic changes in the SCID mouse.

Clinically assessing (through a semiquantitative scoring system like ours) scaliness, erythema, and induration of psoriatic plaques are routinely done in clinical studies. Induration of the graft is a result of increased epidermal thickness but also represents cellular infiltration in the dermis. We adopted this method of assessing the severity of psoriasis to our transplanted grafts, as the grafts on the SCID mice could not be sequentially biopsied. The actual measurements for the clinical assessment were determined by visual inspection and palpation of the grafts at each time point recorded. The clinical parameters are expected, however, to have only weak correlations with histologic measurements, such as epidermal rete lengths and angiogenesis. This is because when the scaliness is severe, the underlying clinical erythema, which is a reflection of angiogenesis and vasodilation, is often masked. One reason the clinical scores may not always have reached statistical significance is the inherent limitations in making a semiquantitative scoring system for this animal model.

In parallel with data from clinical trials, our results indicate that intradermally administered $1,25(OH)_2D_3$ and cyclosporine A can be used to treat plaque psoriasis. Both compounds inhibit IL-2, but cyclosporine A has also been shown to inhibit interferon-γ, a cytokine central to the pathogenic process (Olaniran *et al*, 1996). In contrast, injections of 990 U IL-10 (corresponding to 10^4 U per kg per d) did not cause any detectable decrease in epidermal thickness, but rather produced a slight increase in semiquantitative scores of psoriasis and angiogenesis. Only limited data are available regarding the clinical efficacy of IL-10 in humans (Asadullah *et al*, 1998, 1999; Reich *et al*, 1998). Compared with the recently published data we did not observe any improvement using the PP SCID model. Interestingly, in the aforementioned clinical trial (Asadullah *et al*, 1998), administration of IL-10 led to increased levels of IL-4 and IL-5 derived from circulating T cells. As in the SCID mouse models, additional recruitment of blood-derived T cells in response to intradermal IL-10 was not possible, the IL-10 mediated induction of IL-4 or IL-5 may explain the differences in the clinical results. Alternatively, IL-10 may target a circulating cell present in patients which is not present in the human epidermis/dermis after engraftment in the SCID mouse model (Nickoloff, 1999). It is possible that the key Th2 type cytokine responsible for reversing the Th1 mediated pathology in psoriasis is IL-4 or IL-5. We are particularly interested in IL-4 as it can also inhibit angiogenesis (Volpert, 1998).

Using the PN model we have clearly demonstrated that pharmacologic manipulation of the immune response in psoriatic skin can determine the psoriatic phenotype. Moreover, data from the semiquantitative score of angiogenesis correlated with the clinical data. The fundamental defect in psoriasis has not yet been identified; however, several studies indicate that activation of T cells by superantigens may play an important part (Valdimarsson *et al*, 1997). We decided to activate T lymphocytes from autologous peripheral blood using SEB, because this superantigen is known to activate several T cell receptor Vβ (Leung *et al*, 1995). The induction of the psoriatic phenotype observed, cannot be considered as just a Köebner response, as the injection procedure itself using either several different soluble mediators, or the injection of nonactivated immunocytes has previously been shown not to provoke this conversion of psoriatic phenotype (Wrone-Smith and Nickoloff, 1996; Boehncke *et al*, 1997). The pharmacologic agents studied in the SCID PN model were used in optimal concentrations for *in vitro* inhibition of the cellular immune response or cytokine production, and could only exert their effects after activation of immunocytes and not directly in the skin grafts. We have demonstrated that both $CD4^+$ and $CD8^+$ cells were activated by SEB + IL-2 and the CD4/CD8 ratio remained unchanged after pharmacologic modulation. It is therefore hypothesized that exposure of $1,25(OH)_2D_3$ or cyclosporine A to all ready activated and proliferating lymphocytes results in a selection of T cell clones producing a specific cytokine profile (i.e., Th2 cells) and that these cells once injected in psoriatic skin may serve to deviate the immune response.

Whether topical application of $1,25(OH)_2D_3$ and analogs *in vivo* have any immunosuppressive effect on the antigen-presenting capacity of epidermal cells is still debated (van de Kerkhof, 1995). Calcipotriol-induced improvement in psoriasis, however, has been associated with reduced IL-8 and increased IL-10 levels within lesions (Kang *et al*, 1998).

The advancement in transplantation and cellular immunology have facilitated the development of a psoriasis model in which human skin can be engrafted on to immunodeficient mice (i.e., SCID mice) followed by injection of pathogenic immunocytes. Several groups have confirmed that fully fledged psoriatic plaques can be created using this animal model system, and our data also provide pharmacologic validation of the usefulness of this experimental approach. As many new drugs are being examined for their ability to inhibit inflammation, T cell activation, and, angiogenesis, not only in the skin, but also extracutaneously, this psoriasis model provides a useful animal model to study the response of various human cells maintained in their native configuration in an *in vivo* setting. Future studies are indicated to explore the relevance and utility of this model from a pharmacologic perspective not only with respect to psoriasis, but other T cell mediated processes that share some of the clinical and pathologic factors of psoriasis such as rheumatoid arthritis, Crohn's disease, and multiple sclerosis. It is now possible to determine the efficacy of agents designed to deviate the cellular immune response from a Th1 to a Th2 type in a preclinical setting using entirely human skin and blood-derived immunocytes.

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